Princess Margaret Genomics Centre
101 College Street
PMCRT, Rm 9-601A
Toronto, ON, M5G 1L7

PMGC SAMPLE SUBMISSION GUIDELINES FOR 10X GENOMICS FLEX CLIENTS

Prior to Submission Date

- Please complete the **Sample Submission Form** and send to the PMGC contacts below.
- If your samples require custom probes, please make a note in the **Submission Form** and provide the gene sequences to the PMGC contacts.
- If submitting several Flex assays, please separate your samples into their desired processing groups.
- If submitting fixed single cell/nuclei suspensions dissociated from tissue, please confirm the dissociation method compatibility with 10X Genomics' workflow. Ensure that your dissociation method has been optimized and provides adequate single cell suspensions (sufficient cell counts, ≥95% of the sample is single/unclumped cells with minimal debris) prior to submission.
- If submitting FFPE tissue sections or frozen tissue, please provide an additional sample for dissociation optimization/testing if possible.
  ***NOTE:** If you require additional optimization/testing, please contact Troy Ketela (Troy.Ketela@uhn.ca) for recommendations and associated fees.

Day of Submission

If dropping off samples: Please **schedule your drop off date and time in advance** with your PMGC contact person.

- Your PMGC contact will meet you at the **9th floor elevator lobby** of the Princess Margaret Cancer Research Tower (PMCRT) at your pre-arranged time. PMCRT is the East Tower of the MaRS building, near the corner of College and Elizabeth Street entrance.
- Email or call/text when you are at the designated meeting area and your PMGC contact will come to collect the samples.
- REMINDER: Transport samples using appropriate means of storage (*e.g.* on dry ice for frozen samples, wet ice for fresh samples). Please confirm with PMGC if any questions.

If shipping samples: Please ship out on **Monday/Tuesday** to prevent weekend delays. Place a generous supply of dry ice to ensure dry ice will remain for the duration of the delivery time. For international clients, we recommend shipping with World Courier for tissues/cells. Within Canada, or if shipping DNA/RNA, we recommend FedEX Next Day Priority services.

Shipping address:

Attn: (insert PMGC contact person)
Princess Margaret Genomics Centre
101 College St.
PMCRT, Rm 9-601A
Toronto, Ontario M5G 1L7
Canada

Mary Shi
For 10x Fixed Single Cell inquiries,
(416) 581-7439
Mary.Shi@uhn.ca

Christine Pham
For 10x Fixed Single Cell inquiries,
(416) 581-7439
Christine.Pham@uhn.ca

Dr. Troy Ketela, Head of Operations
For new project inquires,
(416) 634-8816
Troy.Ketela@uhn.ca

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Submission Receiving Conditions (Required)

Fixed/Frozen Single Cell or Nuclei Suspensions

Storage:

- Store the fixed single cell/nuclei suspension(s) using the 10X Genomics -80°C storage guidelines here. Samples can be stored up to 6 months at -80°C.
- Use 1.5mL Lo-bind tubes or equivalent (*e.g.* Eppendorf DNA LoBind Tube 1.5mL Cat#022431021) to minimize sample loss.

Quality:

- Minimum of 300,000 total cells or 500,000 nuclei going into fixation, preferably 1 million cells or nuclei. **DO NOT** exceed 10 million cells/nuclei per fixation reaction.
- Count your cells/nuclei after fixation, before freezing down your samples for submission:
    - **Multiplex**: Ensure a minimum of 200,000 cells or 400,000 nuclei
    - **Singleplex**: Ensure a minimum of 400,000 cells or 800,000 nuclei

  These numbers differ from 10x Genomics' minimum hybridization numbers to account for cell loss during post-storage processing and further downstream processing steps

- **DO NOT** mix samples with different fixation times into one experiment.
- It is recommended that samples are cleaned up if viability is <80%. Low viability may have more variable cell calling and lower sensitivity, potentially leading to poor data quality downstream.
- The Flex assay works best if the sample is fully dissociated before fixation. Please ensure ≤5% cell aggregates in the fixed single cell/nuclei suspensions.
- Debris should be kept to a minimum for best results. Debris can have associated RNA and affect the data quality. Please reach out to confirm if your clean up methods are compatible with 10X Genomics guidelines.

Frozen Tissue

Storage:

- Keep flash-frozen tissue on dry ice in cryovial screw-top tubes (*e.g.* Corning Cryotube with Orange Lid Cat#430488).
- Ideally place tissue loosely in the tube to prevent stacking. Stacking the tissue can potentially jam at the bottom of tube and be inaccessible to remove with tweezers without partial thawing.
- Store fixed tissue pieces using the 10X Genomics -80°C storage guidelines here. Submit on dry ice. Samples can be stored for up to 6 months at -80°C.

Quality:

- Ideally a minimum of 30-50mg. May require more based on cellularity of the tissue:
    - Brain, breast, kidney, liver: 30-50mg
    - Lung, heart, skin, muscle, fat tissue, cartilage: >50mg
- Samples over 5 years from freezing date have been shown to have RNA degradation and this can negatively impact data quality.

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- Samples must not have been any of the following: Freeze-thawed, direct contact with liquid nitrogen, or pre-grounded tissues (if so, please contact us directly).

FFPE Tissue Scrolls

- Obtain and store the FFPE scrolls for processing based on the guidelines here. Scrolls can be stored at **4°C** for up to 1 year.
- On the **Sample Submission Form**, please indicate the date the tissue block was sectioned under "Sample Quality/Collection Date." Please indicate the number of sections x thickness of the scrolls under "Sequencing Requirements and Additional Notes."
- Deliver your scrolls to PMGC at ambient temperature, securing the tubes well to minimize agitation during transport. If scrolls are being shipped to PMGC, a cold pack may be included to mitigate possible temperature fluctuations during transport (as recommended here).
- For human tissue, it is recommended to use two or more 25 µm sections.
- For mouse tissue, it is recommended to use two or more 50 µm sections, depending on tissue type (check the protocol for cell yields from different FFPE tissue types).

Key Considerations

- We recommend performing optimization experiments to validate preparation methods for specific tissues before performing large scale studies.
- Only use reagents recommended by 10X Genomics for the Flex protocol. Please see approved list of Recommended Pipette Tips. You may substitute the formaldehyde for any molecular biology grade stock solution of 37% that is ~19% methanol stabilized with little to no precipitant. See here for more details.
- Each cell suspension/tissue/FFPE block may yield different amounts of material and data quality, depending on different factors (*e.g.* age, pre-storage handling, tissue type, pre-fixation quality, tissue density, size/area of tissue)
- Due to the nature of the 10x Genomics Flex protocol, providing less than the recommended amount of cells/tissue/scrolls may result in significant decrease of target capture number and data output.
- There is a mandatory 30 µm filtration step in the Flex assay protocol. Please be aware of the cell sizes submitted in your sample population.
- For samples known to have red blood cells (RBCs), we recommend performing RBC lysis before fixation of samples. RBC contamination can negatively impact data for single cell sequencing as it can take up sequencing reads.
- For multiplex experiments: we do not recommend pooling samples with different RNA content (*e.g.* PBMC samples with tumor samples, control samples with treated samples if treatment impacts RNA levels). This is because more sequencing reads will be distributed to the samples with a higher proportion of RNA content even though all samples have the same sequencing saturation. Thus, samples with more RNA will have more reads per cell compared with those with lower RNA content. It is not possible to add reads to a particular sample.

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